Author's response to reviews

Title: Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants

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Author's response to reviews: see over
Dear Editor of BMC Complementary and Alternative Medicine,

With reference to manuscript reference number MS: 1509259393768072.

Thank you for sending me the reviewers’ comments. We would like to take this opportunity to submit a revised version of the manuscript in the light of their comments. We hope that this revised version is now suitable for publication.

We have addressed the specific comments as detailed in the following pages.

Thanking you again for your time and looking forward to your reply

Yours faithfully,

Wanchai Assavalapsakul

Department of Microbiology, Faculty of Science,
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Reviewer: DZOYEM Jean Paul

Reviewer notes: I have only a few minor points as follows:

1. Line 93: Authors should precise what medium was used for cell line treatment
2. Line 147: IC50 #250μg/ml considered as effective should be referenced.
3. Line 181: 0.5 McFarland is approximately 1.5x10^8 instead of 1.5x10^6
4. Line 190: Add "Amphotericin B"

Response: Points 1 to 4 have been corrected in the revised version accordingly including a reference has been added in Point 2.
Reviewer: Patrick Bednarski

Major Compulsory Revisions
1. The authors have not determined the antiobesity activity of the compounds per se but simply the lipase inhibitory activity. Whether enzyme inhibition translates into antiobesity activity in vivo has not been determined. Thus, the word “antiobesity” in the title and the text should be replaced by “lipase inhibitory”. The authors can speculate in the text that this activity might translate into antiobesity properties \textit{in vivo}, but this needs to be tested for in animal experiments.

Response: The “lipase inhibitory” is used in place of “antiobesity” throughout the revised version of the manuscript.

2. Along the same lines, a better word than “antitumor”, which implies that the extracts showed activity in animal tumor models (although the word “in vitro” precedes it), would be “cytotoxic”. Then there can be no confusion that the extracts were only tested in cell culture.

Response: The term “\textit{in vitro} cytotoxic” is used in place of “\textit{in vitro} antitumor” throughout the revised version of the manuscript.

3. The first four sentences of the Background (lines 39-44) section should be dropped from the text; the paper is not just about finding anticancer agents but therapies for other disease as well. Furthermore, the 2nd sentence contradicts the 3rd one, i.e., cancer is the leading cause of death in Thailand then it is the third leading cause of death.

Response: They have been deleted from the revised version of the manuscript accordingly

Minor Essential Revisions
Generally, the English is good but the paper would benefit from a work over by a native speaker. Here are some examples:

4. Line 50: Replace “folklore medicine” with “folk medicine”. Folklore are stories passed down from generation to generation by telling them, folk medicine is a therapy passed down through the generations by word of mouth.

5. Line 80: Water is not “dried” but rather “removed” by freeze drying.

6. Line 114: Capitalize “Diphenyl” at the beginning of the sentence

7. Line 213: An “and” is missing between 3.25 and 2.18 μg/ml. Also, a comma should precede and succeed “respectively”.

8. Line 225 and following: Add a space between the numbers and units (e.g. 12.1 μg/ml, not 12.1μg/ml)

9. Line 312: Begin sentence with an “A”, e.g. “A previous study….”

10. Line 350: Change to “Moreover, it could also inhibit growth of …”

Additions to Tables 2 and 3 need to be made:

11. Table 2: Vinblastine with just one “n”.

12. Caption should report that the values are averages with standard deviations of three independent determinations. (see Experimental)

Response: Points 4 to 12 have been corrected accordingly in the revised version, and for Point 12, the caption is now added under the Table 2 in the revised version.
13. What does a dash in Table 3 mean? Not tested? Not determined? Also, the amount of extract tested (2 mg?) in the inhibition zone assay should be reported in the caption. What where the amounts of reference compounds tested for antimicrobial activity with this assay? Couldn’t find this anywhere in the text.

Response: A dash in Table 3 means “No inhibitory activity” and this has been made clear in the Table legend now.

The amount of extract tested is 2 mg in the inhibition zone assay, and is shown on Page 6 Line 173-174 of the revised version but is also now added under Table 3 for clarity as well.
Reviewer: Eduardo Ruiz-Bustos
Reviewer's report:

Major Compulsory Revisions
The manuscript is difficult to read. It is necessary to be reviewed by an English editor.

Response: The manuscript was revised and reviewed by Dr. Robert Douglas John Butcher who is a native English speaking colleague with scientific expertise. He works in Publication Counseling Unit, Chulalongkorn University.

Minor Essential Revisions
Some of the observations are enlisted next:
1. Page 3, line 44: Eliminate space after 2005
2. Page 3, line 67: Change “…were analytical grade.” To “…were of analytical grade.”
3. Page 4, line 76: Change “20 g” to “Twenty grams”
4. Page 4, line 84: Change “…extracts were determined…” to “…extracts was determined” since it refers to the antitumor activity.
5. Page 4, line 94: Remove space between temperature and Celsius degree.
6. Page 4, line 94: Change “…various final concentrations of crude extracts…” to “…crude extracts at different concentrations…”
7. Page 4, line 99: Change “…media were…” to “…media was…”
8. Page 4, line 99: Change “…100 μl DMSO was added…” to “…100 μl DMSO were added…”
9. Page 5, line 103: Change “The inhibition concentration (IC50), the concentration of extract that inhibited cell proliferation by 50%,…” to “The concentration of extract which inhibited cell proliferation by 50% (IC50),…”
10. Page 5, line 108: Change to uppercase the letter “C” as is in the legend below
11. Page 5, line 117: Remove space after “1000 μg/ml) .”
12. Page 6, line 155: Remove space in “namely ; Bacillus”
13. Page 6, line 158: Remove space after yeast
14. Page 6, line 164: Change “…paper discs with the size of 6 mm in diameter…” to “…paper discs with 6 mm in diameter…”
15. Page 7, line 177: Change “…microorganisms with minor modification as previously described in Mothana et al [14]…” to “…microorganisms as previously described in Mothana et al [14], with minor modifications.”
16. Page 7, line 185: Spacing in “…minutes .The change…” to “…minutes. The change…”
17. Page 7, line 185: Change “The change of the colour from yellow to pink…” to “The change from yellow to pink…”
18. Page 7, line 194: Change “The total of 220 extracts…” to “A total of 220 extracts…”
19. Page 7, line 200: Change “among” to “from” and “…only 4 plants namely,…” to “…only 4” and the plant names within parenthesis
20. Page 8, line 204: Avoid the use of the term “obviously”
21. Page 8, line 205: Change “With the value of IC50 at 0.28…” to “With an IC50 value of 0.28…”
22. Page 8, line 207: Change “…also showed IC50 at 1.16…” to “…also showed an IC50 value of 1.16…”
23. Page 8, line 208: Remove space after “fact”
24. Page 8, line 211: Remove space after “pinnata”
25. Page 8, line 214: Remove space after “IC50 of”
26. Page 8, line 224: Change “cytotoxicity” to “citotoxic”
27. Page 8, line 226: Change “…6 plants namely; …” to “…6 plants: …”
28. Page 8, line 234: Remove space after “active”
29. Page 9, line 242: Change “…were tabulated in Table 3.” To “…are shown in Table 3.”
30. Page 9, line 245: Remove the italics in “and”
31. Page 9, line 248: Remove the italics in “and”
32. Page 9, line 252: Remove the italics in “and”
33. Page 9, line 256: Remove the italics in “and”
34. Page 9, line 257: Add space in “E.coli”
35. Page 9, line 261: Remove the italics in “and”
36. Page 10, line 277: Remove extra space after “Thailand,” and change “…the total…” to “…a total…”
37. Page 10, line 280: Remove “Most of them did not show any antitumor activity except some of the extracts.” Is repetitive
38. Page 10, line 287: Remove space after “Clinically”
39. Page 10, line 289: Change “agents” to “agent”
40. Page 11, line 317: Change “…control the cell division.” To “…control cell division.”
41. Page 11, line 320: Change “…only the extracts from ethanol and aqueous…” to “…only the ethanol and aqueous extracts…”
42. Page 11, line 327: Change “…which are the rich sources…” to “…which are rich sources…”
43. Page 12, line 354: Change “…of the leaves…” to “…from the leaves…”
44. Page 12, line 362: Add space within “E.coli”

Response: Points 1 to 44 have been corrected accordingly
Reviewer: Maria Leticia Miranda Fernandes Estevinho

Reviewer's report:
The article is not very original, but the results are interesting and the writing style is adequate, however, some concerns arise. The article must undergo major revisions.

Response: The concept of using folk medicine as a guide to plant species and within that to plant tissue selection for assaying for bioactive components, as with the methods used, is indeed not novel but also not that well established. However, the database of these 52 species contains newly assayed plant species and establishes the merit of such an approach. In addition the manuscript anecdotally highlights some of the pitfalls of screening crude extracts (e.g. residual lipid extraction means such crude low polarity fractions are not of use in anti-lipase assays, and bulk solubility is an issue in aqueous viability assays where the crude extract may require a greater non-polar solvent (like DMSO) concentration to remain soluble than the lethal limit to the target microbes

The abstract is too large and must be summarized.
Response: The abstract has been rearranged and summarized accordingly.

The section “Background” must be improved, since it is incomplete. It would be very interesting to provide information about the use in ethomedicine of the medicinal plants used (as they are many, refer the most important and justify the choice of the others). “Since a number of plants have been known to provide the basis of traditional medicine for a long time, a significant number of novel metabolites with potent pharmacological properties have recently been discovered from plants.” Add a reference to this sentence.

Response: Some references have been added to the revised version accordingly.

Provide more complete information about the objective of the present study; refer the existence of previous studies.
Response: Further information has been included in the Background section in the revised version (Page 2 Line 50-56)

In the “Methods” section give more details about the taxonomic identification of the medicinal plants.
Response: The taxonomic identification was performed by Metropolitan Electricity Authority (MEA) from the Mangrove Forest Restoration and Regeneration Project during 2004 to 2008. Therefore, we cannot access the methodology used to identify plants further, but voucher specimens are available.

Explain better the MTT cytotoxic assay.
Response: The MTT cytotoxic assay method has been modified in the revised version accordingly.

In the discussion, give more details about “This finding suggested that the extracts from these two species of the family Sonneratiaceae can possibly be further developed as the promising antimicrobial agents.”.
Response: Further details are now provided in the “Discussion” in the revised version (Page 12 Line 387-401).
Justify conveniently: “Unfortunately, the dried hexane and dichloromethane extracts could not be dissolved in the media for the tests, only the ethanol and the aqueous extracts were obtained for the tests.”

Response: The dried hexane and dichloromethane extracts were dissolved in a low polarity solvent (DMSO) first and then diluted into the nutrient broth. However, at final concentrations of DMSO that alone did not affect the test bacterial culture viability (5% (v/v)) the crude extracts were not fully soluble and thus could not be reliably assayed. This has now been briefly explained in the manuscript.

“For the antioxidant activity, only the extracts from ethanol and aqueous from 6 investigated plants showed effective free radical scavenging activity”.

Response: All four crude extracts (hexane, dichloromethane, ethanol and water) were tested but only crude ethanol and water extracts from some of these (6 species) showed any free radical scavenging activity. This has been made more clear in the revised version.

“For the antiobesity activity, only ethanol and water extracts were used to study for the pancreatic lipase inhibition.” Why?!

Response: The low polarity hexane and dichloromethane solvents also extract some natural lipids from the plant tissue and these were found to be at high enough levels to interfere with the assay through acting as a substrate for lipase. Because the amount and composition of the lipids in each extract is unknown and likely to vary, this cannot easily be accounted for in control assays and, therefore, they were not be used for this study as all positive results could be false positives whilst weak and even moderate true positive results could be masked. Thus these crude extracts need further fractionating to remove the lipid components prior to assay. A brief explanatory statement has now been added in the revised manuscript.

In the conclusions, add a paragraph about the future application of this study.

Response: Subject to within the limitations of not over-interpreting the results, this has now been extended accordingly.”

A global revision of the language, to correct the grammatical errors, must be performed.

Response: The revised manuscript has been revised and reviewed by Dr. Robert Douglas John Butcher who is a native English speaking colleague with scientific expertise. He works in the Publication Counseling Unit, Chulalongkorn University.

A statistical treatment must be applied to the results.

Response: Some statistical values have been added into the results of the revised manuscript.